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## ILSI Health and Environmental Sciences Institute

January 24, 2005

Dear Dr. Sir or Madam:

The ILSI Health and Environmental Sciences Institute's (HESI) Protein Allergenicity Technical Committee is pleased to provide the accompanying comments on FDA's draft guidance document entitled "Guidance for Industry: Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use."

The Committee has been engaged in number of activities over the past few years to advance the science associated with the development of reliable and accurate methodologies to characterize the allergenic potential of novel proteins. ILSI HESI is a global branch of the International Life Sciences Institute, a public, non-profit scientific foundation with branches throughout the world. ILSI HESI provides an international forum to advance the understanding and application of scientific issues related to human health, toxicology, risk assessment and the environment. ILSI HESI is widely recognized among scientists from government, industry and academia as an objective, science-based organization within which important issues of mutual concern can be discussed and resolved in the interest of improving public health. As part of its public benefit mandate, ILSI HESI's activities are carried out in the public domain, generating data and other information for broad scientific use and application, and include participation from government, industry, and academic scientists.

As a United Nations recognized non-governmental organization (NGO), ILSI HESI has provided input on the development of a number of international guidance documents associated with novel protein evaluation including the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology and the report from the Joint Food Agriculture Organization (FAO) / World Health Organization (WHO) Expert Consultation on Foods Derived from Biotechnology. In addition, the committee has provided comments to the Japanese Food Safety Commission and the US Food and Drug Administration on the development of safety assessments for novel proteins.

HESI has worked actively with FDA on a number of these activities and we are extremely encouraged by the extent to which the Agency is committed to the use of sound science to meet its mission to protect public health. Feel free to contact me directly if you have any questions regarding these comments.

Sincerely,

Karluss Thomas  
Sr. Scientific Program Manager, HESI

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Protein Allergenicity Technical Committee – January, 2005

**Comments on the FDA draft guidance document: "Guidance for Industry: Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use."**

## Background

The FDA has provided a draft guidance document resulting from a request from the U.S. Office of Science and Technology Policy (OSTP) to update field test requirements and to establish early voluntary food safety evaluations for new proteins produced by bioengineered plants (August 2, 2002; 67 FR 50578). Consistent with the Coordinated Framework for the regulation of Biotechnology Products (June 26, 1986; 51 FR 23302), the FDA is requesting an initial set of data for all products expressing a novel protein that has not been previously reviewed by the agency with the exception of PIPs, which are regulated by EPA. We concur with the FDA view that any potential food safety concerns, at this early stage of development, should be limited to potential allergic reactions in susceptible people or toxic reactions in people or animals that might occur due to the presence of the introduced protein. Such a preliminary analysis should focus on factors that are clearly correlated with risks of allergenicity or toxicity and would help to ensure public safety due to even limited exposure that might arise from limited scale production of the crop prior to a full safety assessment.

Estimates for the incidence of food allergy affect approximately 4% of the total population (Sampson, 2004). However, severe allergic reactions (anaphylaxis) to foods are relatively rare, occurring in approximately 3.2 individuals per 100,000 people per year (Burks and Sampson, 1997). Anaphylaxis to food typically occurs in those who are genetically predisposed to allergy, have been previously sensitized to the allergen and who unintentionally consume the allergen (Barnes, 2000; Sicherer, 2000). A small number of specific proteins contained in relatively few foods are responsible for most severe reactions and these are mediated by allergen specific IgE (Bush and Hefle, 1996; Eigenmann and Sampson, 1997). At this time, there is no cure for food allergy and the disease must be managed through avoidance of the allergenic food (Smith and Munoz-Furlong, 1997). Food labeling laws for processed foods in the U.S. provide significant protection for allergic individuals by requiring the identification of all ingredients, especially those derived from commonly allergenic foods. Ingredient labeling allows those with food allergies to avoid the foods that can cause them harm. Consequently, an important public health consideration for foods or processed fractions derived from genetically modified crops is the need to protect food allergic subjects from unwanted exposure to allergens that cause their disease. In practice, this means that the risk assessment must assure that no known or likely allergen, especially food allergens (but including all

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known allergens) will be transferred or “hidden” in foods or fractions derived from genetically modified crops (Metcalfe *et al.*, 1996).

Protein safety testing, in the context of overall safety testing for biotech crops, utilizes many different sources of information. An analysis of a history of safe use and the mode of action of a protein provides the first level of information. If the protein, or a structurally and functionally related protein, is already in the diet, the concerns are minimized. Absence of a history of safe use does not indicate that a protein is a hazard, but does require that some basic testing needs to be undertaken. The potential for unexpected alteration of nutrient composition in the new food or including an inherently toxic protein should be avoided. The better the mode of action is understood, the less likely it is that the protein will cause some unexpected effect. An understanding of the mode of action is also important in developing a testing strategy.

### **History and Introduction**

Prior to the marketing of foods derived from crops modified through biotechnology, developers of these products are required, through a variety of national and international regulatory authorities, to evaluate their safety. One element of the safety assessment includes consideration of the potential allergenic activity of the protein(s), which are produced from the introduced genes. To date, approximately 70 genetically modified products have been assessed for safety and approved for food use in at least one country by regulatory authorities (AGBIOS, 2001). The process by which the safety assessment has been conducted has included guidance from a variety of expert scientific bodies, including the US FDA, (1992, 1994), a joint US FDA, EPA and USDA Panel (1994), the European Commission, (EC Directive, 1995), ILSI AII/IFBC (1996), FAO/WHO (1996, 2000), and Codex (2000, 2002, 2003).

During the product development cycle, early field trials are performed to evaluate a wide range of events to ensure that the trait is expressed as desired and that agronomic performance is acceptable. These trials are generally small in scale and performed under the auspices of the USDA regulations (7 CFR 340). The USDA requires measures for isolation to minimize pollen flow and the risk of new traits entering the food/feed stream. In addition, a bioinformatics analysis to assess similarities to potential allergens and toxins is done for all new proteins prior to environmental release.

### **Early Food Safety Evaluation of New Non-Pesticidal Proteins**

The FDA draft guidance for early food safety evaluation outlines seven elements of scientific evaluation that are a subset of the usual biotechnology consultation with the FDA about a food derived from a new bioengineered plant variety. It is understood that the submission of this early food safety evaluation does not replace the need for the more detailed consultation prior to commercial release of a new biotechnology plant variety.

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Efforts are currently underway at several organizations including the International Life Sciences Institute Health and Environmental Sciences Institute (ILSI HESI), the European Center for the Evaluation of Alternative Methods (ECVAM), and others, to evaluate and validate specific methods and criteria to be used to perform amino acid sequence searches and to standardize the pepsin digestion assay. It is in this area of validation that we believe is the biggest challenge to the early food safety evaluation of new non-pesticidal proteins.

A list of the elements described by the FDA in their guidance document is provided below along with comments on the technical feasibility:

1. The name, identity, and function of the new protein(s)
2. History of safe use of the new protein(s)
3. A list of identity(ies) and source(s) of the newly introduced genetic material
4. A description of the purpose or intended technical effect of the new protein(s)

The information from the first four areas described above is readily available at the very earliest stages of product development. The only possible exception is a full understanding of the mode-of-action of a particular trait. While the intended technical effect or purpose is known, field releases are generally required to confirm proof of concept.

5. An assessment of the amino acid similarity between the new protein and known allergens and toxins. The purpose is to identify any sequence similarities that might indicate possible allergenic cross-reactivity to known allergens or toxins which are present in public databases. If significantly similar to a known allergen, further testing to evaluate potential cross-reactivity (e.g., by using IgE binding studies) would be warranted. Likewise, if a potential similarity to a known toxin is identified; appropriate animal testing for toxicity would be advised.

Is the amino acid sequence similar to any known allergens or toxins? Searches are typically conducted by using FASTA or BLAST algorithms to identify overall homology of the introduced protein to known cross-reactive allergens and toxins with similar modes of action. In addition, a short, identical amino acid search algorithm is performed to identify any short regions of identity. Current matching criteria include 8 or more contiguous amino acids that are identical to any segment of any known allergen (food, inhalant or contact allergen) or greater than 35% identity over an 80 amino acid segment of a protein. Any protein that exceeds these criteria precipitates the need for further testing. Empirical observations of allergen cross-reactivity suggest that proteins with overall identities of

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greater than 70% are likely to be cross-reactive, while those of less than 50% identity are unlikely to cause cross reactions (Aalberse, 2000). It is also clear however, that segments of short matches or low identities are unlikely to represent cross-reactive proteins (Hileman et al., 2002, Goodman et al., 2002).

In February 2005, the ILSI HESI Protein Allergenicity Technical Committee is sponsoring an expert workshop and monograph on this topic. We encourage participation of the FDA in this workshop.

6. The overall stability of the protein (i.e. temperature, processing, etc.), and the resistance of the protein to enzymatic degradation using appropriate in vitro assays.

Is the protein stable to digestion in pepsin or other enzymes?  
Current validation testing to standardize assay parameters and evaluate specific variables including differences in the pH of the assay (1.2 vs. 2.0) has been recently completed. The ILSI HESI Protein Allergenicity Technical Committee coordinated an international ring study of nine laboratories (Thomas et al., 2004). The following conclusion was made: "These data demonstrate that this common protocol for evaluating the in vitro digestibility of proteins is reproducible and yields consistent results when performed using the same proteins at different laboratories." As a next step we are seeking ISO certification for this protocol.

It should also be recognized that some products under development may not include the expression of a new protein therefore other endpoints may need to be identified.

### **The FDA has requested comments of the following four topics:**

1. Whether the proposed collection of information is necessary for the proper performance of the FDA's functions, including whether the information will have practical utility.

We agree with the FDA that the greatest risk is related to the issue of potential allergenicity and toxicity of new non-pesticidal proteins. The best approach combines basic information about the genes and proteins introduced into the plant and from bioinformatics and stability data on the new protein. As stated above, this is but a subset of information that is provided through the usual consultation process and as such can be readily obtained at this earlier stage of development.

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2. The accuracy of the FDA's estimate of the burden of the proposed collection of information, including the validity of the methodology and assumptions used.

We agree with the FDA's estimated burden of the collection of information. The methodology chosen is scientifically based and also fully supported by the international community, such as Codex.

3. Ways to enhance the quality, utility, and clarity of the information to be collected.

We believe that the quality, utility and clarity of the information are best gained through the use of international validated methodology. Our mission over the last few years has been to advance the scientific understanding of relevant parameters for characterizing the allergenic potential of novel proteins and biotechnology products. To that end, we successfully showed the robustness of a pepsin digestibility protocol across nine international laboratories (Thomas et al., 2004). In addition to gaining publication in a peer-reviewed scientific journal, we also are seeking AACC validation and ISO certification for the method. A similar process is being used to prepare standard methodology for a bioinformatics tool. A monograph will be developed as a part of the workshop that HESI is sponsoring in Feb 2005.

4. Ways to minimize the burden of the collection of information on respondents, including through the use of automated collection techniques, when appropriate and other forms of information technology.

It may be possible to provide an efficient method of data presentation, collection and review if standard forms or formats are provided for certain required elements, such as a bioinformatics report, or the pepsin resistance assay. Further efficiencies may be gained by electronic submission of data.

### **Conclusion**

The process by which the early safety assessment of proteins produced from genes transferred into food crops through biotechnology has been conducted through a science-based step-wise, weight of evidence approach that was developed with guidance from a variety of expert scientific and regulatory bodies. While the process of evaluating the safety of new varieties of crops produced using the tools of biotechnology is voluntary, it should be noted that all such products have been evaluated prior to market introduction, following the guidance of the FDA originally outlined in 1992. Since the FDA has the authority to request safety data on food products, and the responsibility to ensure that unsafe foods are removed from the market place, the biotechnology industry considers the guidance regarding the safety assessment as mandatory.

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